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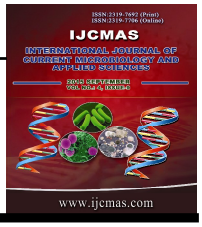

Original Research Article

Use of Serological markers for evaluation of patients with Rheumatoid arthritis

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Keywords

Antinuclear antibody, Indirect immuno-fluorescence, Anti-cyclic-citrullinated peptide antibody, Enzyme Linked Immunosorbent assay, Rheumatoid factor, Latex agglutination assay

A B S T R A C T

The aim of this study was to determine the frequency of antinuclear antibody, rheumatoid factor, and anti-cyclic citrullinated peptide antibody in patients with rheumatoid arthritis and to compare anti-cyclic citrullinated peptide antibody with rheumatoid factor. This prospective study was carried out for a period of two months in a tertiary care hospital. In this study, 85 blood samples were collected from arthritis patients. For all we measured the antinuclear antibody (Indirect immunofluorescence), cyclic-citrullinated peptide (Enzyme Linked Immunosorbent assay) and rheumatoid factor (Latex agglutination assay). Out of 85 patients with arthritis, 51(60%) were females and 34(40%) were males. Median age was 57.5 years for males and 48.5 years for females. Eighty five patients were diagnosed clinically as rheumatoid arthritis. The prevalence of antinuclear antibody, anti-cyclic citrullinated peptide antibody and rheumatoid factor were 4.7% (4), 48.2% (41) and 10.6% (9) respectively. Number of cases both positive for rheumatoid factor and Anti-CCP is seven. Three cases were positive for ANA, rheumatoid factor and Anti-CCP. Females have a greater predisposition for Rheumatoid arthritis than males. Fourteen patients with positive results for Anti-CCP had high concentration (100 IU) whereas 27 patients had low concentration (6.25- 50 IU). Two patients showed a high RF titre (≥ 1/128), seven of IgM-RF positive patients had low titre (1/16- 1/64). Only four patients of 85 were positive for antinuclear antibody, of them three had high titre (> 1/160) with speckled pattern and one had 1/80 titre with cytoplasmic pattern. For evaluation of patients with suspected rheumatoid arthritis recommended to perform cyclic-citrullinated peptide antibody and IgM- rheumatoid factor. The results of both tests are informative, since a positive result for either test increase diagnostic sensitivity, while the specificity is increased when both tests are positive.

Introduction

Rheumatoid arthritis is a chronic, severe, progressive inflammatory autoimmune disease of uncertain aetiology involving multiple peripheral joints (Van Boekel *et al.*, 2001). Rheumatoid arthritis can also produce diffuse inflammation in the lungs,

pericardium, pleura, and sclera, and also nodular lesions, most common in subcutaneous tissue. About 1% of the world's population is afflicted by Rheumatoid arthritis, and is much more common in women than in men and generally occurs between the ages of 40 and 60 years.

The disease onset is usually gradually, with the predominant symptoms being pain, morning stiffness and swelling of many joints (Lee and Weinblatt, 2001). Early tends to affect smaller joints of hand and feet and later on as the disease progresses, symptoms often spread to the knees, ankles, elbows, hips and shoulders (Fleming *et al.,* 1973; Jacoby *et al.,* 1973). Rheumatoid arthritis is diagnosed according to clinical findings and serologic testing.

The main useful serological markers are rheumatoid factors and antibodies to citrullinated peptides. Rheumatoid factor (RF) is IgM autoantibody directed against the Fc portion of IgG. It is found in 75 to 80% of rheumatoid arthritis patients, but has a low specificity because it may be found in healthy elderly individuals and patients with other autoimmune diseases or infections (Bartfeld, 1960; Mikkelsen *et al.,* 1967). Anti cyclic Citrullinated peptide antibody (Anti-ccp) is autoantibody that bind antigenic determinant of unusual amino acid citrulline formed by posttranslational modification of arginine residues. The sensitivity of Anti-ccp for RA varies from about 50% to 75%, while specificity is relatively high, usually over 90% (Schellekens *et al.,* 1998; Nishimura *et al.,* 2007). ANA testing is also done to rule out Systemic Lupus Erythematosus.

The present study was undertaken to determine which serological marker is useful to diagnose rheumatoid arthritis and to compare cyclic citrullinated peptide antibody with rheumatoid factor.

Material and Methods

This prospective study was carried out in Microbiology Department, Tirunelveli Medical College Hospital, Tamil Nadu, India from April 2014 to September 2014.The study protocol was approved by the ethical committee of the institution. The patients gave written informed consent to participate in the study. Blood Samples obtained in the Microbiology Department were separated into sera and stored at - 20°C until for serology testing.

All laboratory works were undertaken in the Microbiology laboratory. Sera were subjected to serological tests like RF, Anti-CCP and ANA-IF. Anti-nuclear Antibody was determined quantitatively by indirect immunofluorescence assay using Bio systems HEp-2 cell line substrate reagent kits. Anti-cyclic citrullinated peptide antibodies were determined quantitatively by Enzyme-Linked Immunosorbent assay (ELISA) using Genesis Diagnostics (UK). Rheumatoid factor -IgM was determined qualitatively and semi-quantitatively by latex agglutination assay using Agappe Diagnostics, Kerala.

Results and Discussion

Out of 85 patients with arthritis, 51(60%) were females and 34(40%) were males. Median age was 57.5 years for males and 48.5 years for females. Eighty five patients were diagnosed clinically as rheumatoid arthritis. The prevalence of antinuclear antibody, anti-cyclic citrullinated peptide antibody and rheumatoid factor were 4.7% (4), 48.2% (41) and 10.6% (9) respectively (Figure 1 & 2).

In this study, the number of RF positive cases is 9, of which one was male and 8 were females. Most of RF positive patients were in the age group of 21–60 (Table 1). Number of Anti-CCP positive cases is 41 of which 14 were males and 27 were females. Most of Anti-CCP positive patients were in the age group of 20–60 (Table 2). Number of cases both positive for rheumatoid factor and Anti-CCP is 7 of which one was male and six were females. Three females were positive for ANA, Rheumatoid factor and Anti-CCP. Females have a greater predisposition for Rheumatoid arthritis than males. Positivity for RF and Anti-CCP test was greater for women than for men.

Fourteen patients with positive results for Anti-CCP had high concentration (100 IU) whereas 27 patients had low concentration (6.25- 50 IU) (Table 3). Two patients showed a high RF titre (≥ 1/128), 7 of IgM-RF positive patients had low titre (1/16-1/64). Only 4 patients of 85 were positive for antinuclear antibody, of them 3 had high titre (> 1/160) with speckled pattern and one had 1/80 titre with cytoplasmic pattern.

In recent years it has become clear that early aggressive treatment in rheumatoid arthritis (RA) reduces joint damage and improves function. To use a potentially toxic therapy as early as possible we require an accurate diagnosis of RA and also information about prognosis in an individual patient. Apart from clinical features, auto antibodies progressive joint damage reflected in radiographs by bony erosion and as joint space narrowing. Since structural joint damage is irreversible, early recognition and treatment are currently being emphasized, with the goal of halting progression of the disease.

The early diagnosis of rheumatoid arthritis is important to prevent crippling. So it is important to diagnose RA at a very early stage in the disease, when often not all clinical symptoms are manifest, so good serological markers are needed (Vossenaar and Venrooij, 2004).

When RA is clinically suspected, immunological studies are required, such as testing for the presence of rheumatoid factor and anti-cyclic citrullinated peptide. RF was the first autoantibody detected in patients with RA. It was discovered in the early twentieth century and became the primary serological test used in the diagnosis of RA (Renaudineau *et al.*, 2005). Rheumatoid factor also found in many other diseases, including Sjogren's syndrome, systemic lupus erythematosus, mixed connective tissue disease, chronic infection and in healthy elderly population.

Table.1 RF – age/sex wise distribution

Age Group	No. of males	No. of females
<20	-	-
21-40	-	2
41-60	1	5
61-80	-	1
>80	-	-

Table.2 Anti CCP –Age/Sex wise Distribution

Age group	No. of males	No. of females
<20	2	3
21-40	7	13
41-60	3	8
61-80	2	2
>80	-	1

Table.3 Anti-CCP positivity according to titre value

Cut off value	No of positives
6.25 IU	1
12.5IU	11
25 IU	10
50 IU	5
100 IU	14

Figure.1 Prevalence of serological markers in rheumatoid arthritis patients

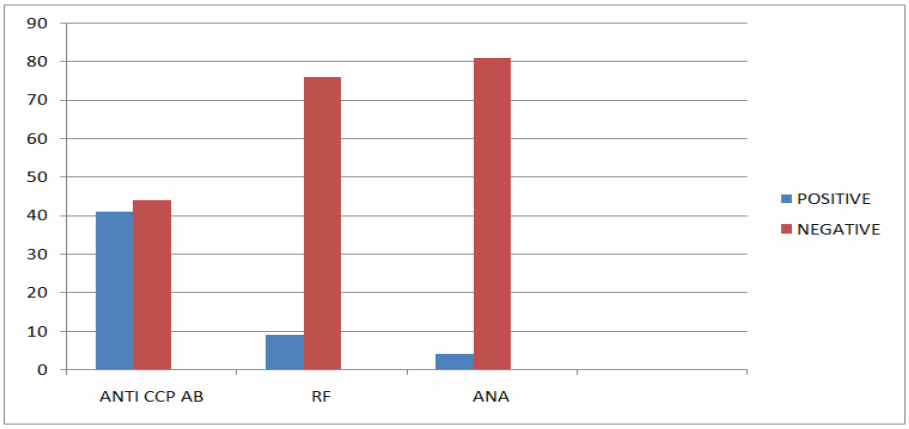
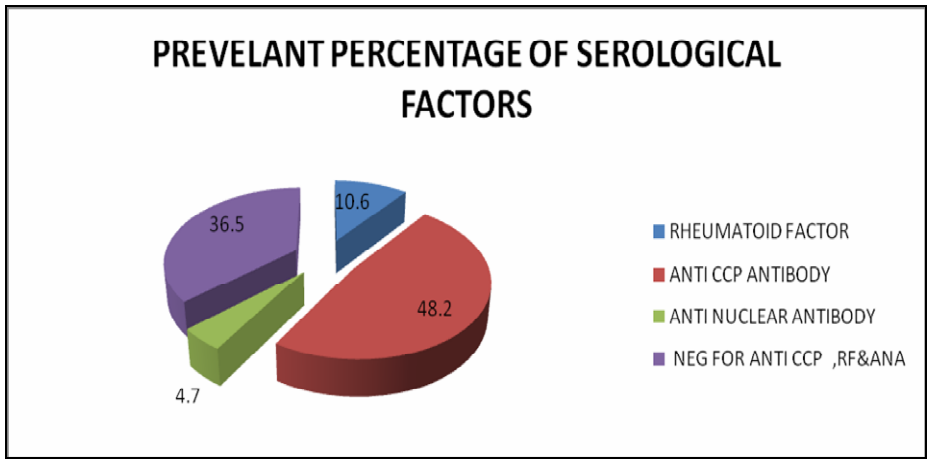


Figure.2 Prevalent percentage of serological factors



Because of this low specificity, new serological test such as anti- cyclic citrullinated protein antibodies is used for diagnosing rheumatoid arthritis. The detection of anti-CCP is useful for the diagnosis of RA because of its similar sensitivity but higher specificity compared with RF. In the present study, the diagnostic value of anti-cyclic citrullinated peptide (anti-CCP) antibodies and other potential diagnostic biomarkers such as IgM rheumatoid factor and ANA was evaluated for predicting early development of rheumatoid arthritis (RA).

In the present study, high prevalence of anti-CCP antibodies (48.2%) was found in patients with rheumatoid arthritis than RF (10.6%) and also low prevalence of antinuclear antibodies (4.7%) with low titre was found. This is important to rule out systemic lupus erythematosus. The combination of two biomarkers (RF and CCP) together provide important diagnostic tool about rheumatoid arthritis.

For evaluation patients with suspected rheumatoid arthritis recommended perform cyclic-citrullinated peptide antibody and IgM- rheumatoid factor. The results of both tests are informative, since a positive result for either test increase diagnostic sensitivity, while the specificity is increased when both tests are positive.

Anti-CCP antibodies may serve as a better diagnostic marker than RF in Indian population, especially in patients with arthritis of unclear aetiology. Usefulness of this assay largely rests in distinguishing RA from other rheumatic disorders and recommends performing cyclic-citrullinated peptide antibody and IgM- rheumatoid factor for evaluation of patients with suspected rheumatoid arthritis. The anti-CCP2 assay has proved to be very helpful and seems to now the diagnostic marker of choice for the diagnosis of RA especially early cases.

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References

Bartfeld, H. 1960. Incidence and significance of seropositive tests for Rheumatoid factor in non-rheumatoid disease. *Ann. Intern. Med*., 52: 1059–1066.

Fleming, A., Crown, J.M., Corbett, M. 1973. Early rheumatoid disease. I. Onset. *Ann. Rheum. Dis*., Pp. 357.

Jacoby ,R.K, Jayson, M.I., Cosh, J.A.1973. Onset, early stages, and prognosis of Rheumatoid arthritis: a clinical study of 100 patients with 11-year follow up. Br J. 2:96.

Lee, D.M., Weinblatt, M.E. 2001. Rheumatoid arthritis. *Lancet*, 358: 903.

Mikkelsen, W.M., Dodge, H.J., Duff, I.F., Kato, H. 1967. Estimates of the prevalence of rheumatic diseases in the population of Tecumseh, Michigan, 1959–1960. *J. Chronic. Dis*., 20: 351–369.

Nishimura, K., Sugiyama, D., Kogata, Y. *et al.* 2007. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann. Intern. Med*., 146: 797.

Renaudineau, Y., Jamin, C., Saraux, A., Youinou, P. 2005. Rheumatoid factor on a daily basis. *Autoimmunity*, 38(1): 11–6.

Schellekens, G.A., de Jong, B.A., van den Hoogen, F.H. *et al.* 1998. Citrulline is an essential constituent of antigenic determinants recognized by Rheumatoid arthritis-specific auto antibodies. *J. Clin. Invest.*, 101: 273.

Van Boekel, M., Vossenaar, E.R., van den Hoogen, F.H.J., van Venrooij, W.J. 2001. Autoantibody systems in rheumatoid arthritis: sensitivity and diagnostic value. *Arthritis. Res.,* 4: 87–93.

Vossenaar, E.R., Venrooij, W.J.V. 2004. Anti- CCP antibodies, a highly specific marker for (early) rheumatoid arthritis. *Clin. Appl. Immunol. Rev*., 4(4): 239–262.